Scanning Temperature Gradient Focusing

This project is part of an ongoing effort to develop new microfluidics-based technologies for chemical and biochemical analysis. In order to realize the promise of microfluidic technologies for fast, portable, and inexpensive analyses, robust methods for sample concentration in small-volume microchannels are required. Temperature gradient focusing (TGF) is a technique, recently developed at NIST, that can be used for the simultaneous concentration and separation of a wide range of biomolecular analytes.

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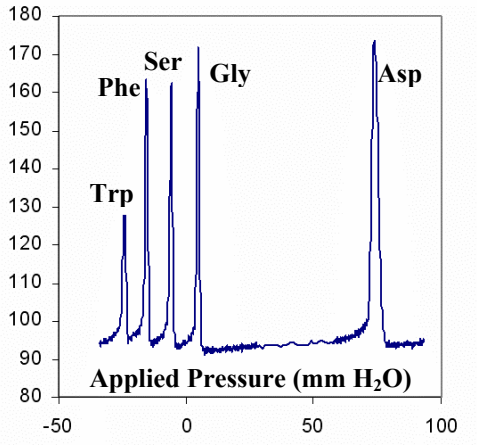
Temperature gradient focusing (TGF) works by balancing the electrophoretic motion of an analyte against the bulk flow of buffer through a microchannel or capillary. A temperature gradient is applied along the length of the channel, and a buffer with a temperature-dependent ionic strength is used to create a corresponding gradient in the electrophoretic velocity of the analyte. Consequently, the bulk flow velocity and the electrophoretic velocity will sum to zero only at a single point along the gradient and all of the analyte will move toward that zero velocity point where it will accumulate or focus. Different analytes with different electrophoretic mobilities will focus at different points and are thereby separated.

One potential drawback to TGF is the limited peak capacity; only a small number of analyte peaks (≈ 2 to 3) can be simultaneously focused and separated. For many applications, it is necessary to separate and quantitate a large number of components from a complex sample mixture. In this work we developed a new method that can be used with TGF to provide separations with both high resolution and high peak capacity in short microfluidic channels.

The new method, scanning TGF, was demonstrated to enable high-resolution separations of amino acids as shown in the figure. The use of scanning TGF effectively injects and focuses each analyte for a controlled amount of time; separations are more repeatable and quantitative. Peak areas (which are proportional to the sample concentration) have a typical reproducibility of 10% or better. In addition, it simplifies the detection scheme required for the technique, making it compatible with single-point laser-induced fluorescence (LIF) and ultraviolet (UV) absorbance detection typically used with capillary electrophoresis (CE).

The technique was demonstrated with AC conductivity detection for the separation and detection of metal cations. Finally, it was demonstrated that scanning TGF could be used to adjust the detection limits as needed for a given sample.

The Figure Shows a Scanning TGF Separation of Five Dansylamino Acids, Where the UV Signal Intensity is Plotted vs the Applied Pressure.



The applied pressure was scanned from 93 mm H_2O to 58 mm H_2O at a rate of about 3 mm/min and from 28 mm H_2O to -34 mm H_2O at a rate of about 1.5 mm/min. The intensity was monitored as a function of the applied pressure at a fixed point near the end of the gradient zone.

This research has improved NIST's capabilities for separation and detection of complex samples in portable and inexpensive microfluidic systems.

Future Plans:
Further work on TGF and related technologies is ongoing. Scanning TGF has become a standard operating procedure in our lab.

Publications:

Ross, D.J.; Balss, K.M.; Hoebel, S.J.; Jones, B.J.; Malliaris, C.; Vreeland, W.N. ***"Scanning Temperature Gradient Focusing for Simultaneous Concentration and Separation of Complex Samples"***, In *Proceedings of the μTAS Conference Volume 2,* Jensen, K.F., Han, J., Harrison, D.J., Voldman, J. Eds.; TRF, Inc., San Diego, 2005; pp. 1022-1024.